

藏药黑边假龙胆的化学研究

张颖君 杨崇仁*

(中国科学院昆明植物研究所植物化学开放研究实验室, 昆明 650204)

摘要 龙胆科植物黑边假龙胆(*Gentianella azurea* (Bunge) Holub)是治疗肝胆湿热的1种藏药,从西藏日喀则产的全草中分离得到9个化合物,其中1个C-甾黄酮,异荭草素(isoorientin);1个吡喃酮甙,獐牙菜酚甙(swertianolin);1个木质体甙,橄榄脂素 4-O- β -D-葡萄糖甙(olivil 4-O- β -D-glucoside);3个环烯醚萜甙,龙胆苦甙(gentiopicroside),獐牙菜甙(swertiamarin)和马钱子酸(loganic acid);1个三萜,齐敦果酸(loeanolic acid);1个甾醇, β 谷甾醇葡萄糖甙(daucosterol)以及1个长链脂肪醇,三十烷醇(n-1-triacontanol)。它们的结构通过光谱和化学的方法得到了鉴定。上述结果,从次生代谢产物的角度为假龙胆属在系统分类上与龙胆属和獐牙菜属有着密切的联系提供了旁证。

关键词 黑边假龙胆, 龙胆科, C-甾黄酮, 化学分类

CHEMICAL STUDIES ON GENTIANELLA AZUREA, A TIBETAN MEDICINAL PLANT

ZHANG Ying-Jun, YANG Chong-Ren*

(Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204)

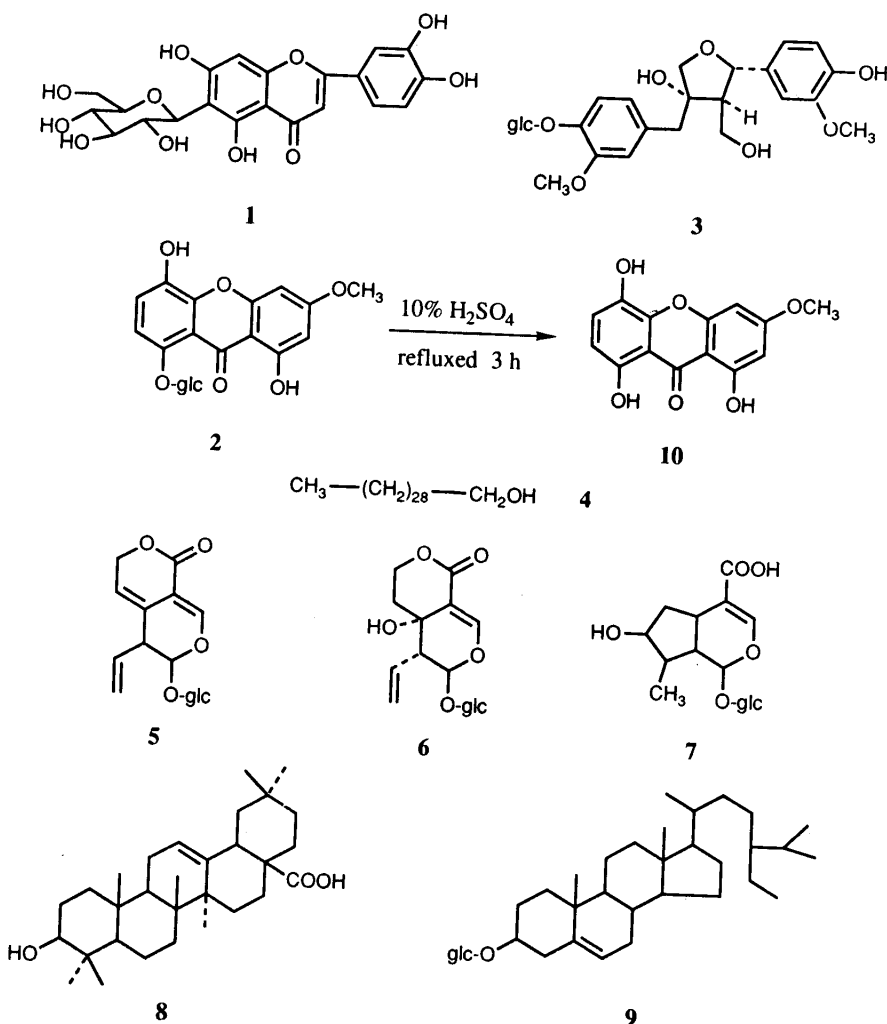
Abstract Nine compounds, which include a C-glucosyl flavonoid, isoorientin; a xanthone glycoside, swertianolin; a lignan glycoside, olivil 4'-O- β -D-glucose; a stright chain fatty alcohol, n-1-triacontanol; three iridoidal glycosides, gentiopicroside, swertiamarin and loganic acid; a triterpenoid, oleanolic acid and a sterol, daucosterol were isolated from the whole herbs of *Gentianella azurea*. The structures of these compounds were determined by means of spectral and some chemical evidence.

Key words *Gentianella azurea*, Gentianaceae, C-glucosyl flavooid, Chemotaxonomy

Gentianella azurea (Bunge) Holub, as a gentianaceous herb, is widely distributed in the west China^[1]. It was used as a drug in Tibetan traditional medicine for substantial heat in the liver and the gallblader. *Gentianella* as a big genus of Gentianaceae is closely related with *Gentiana*. Up to now, there are not more chemical studies for this genus yet. As a part of studying on the chemistry of gentianaceous medicinal plants and traditional Tibetan medicinal plants, we investigated the chemical constituents of the whole herbs of the this plant and obtained nine compounds. In this paper we report here the isolation and the structural determination of these compounds.

MeOH extracts of dried whole herbs of *Gentianella azurea* was suspended in H_2O and then successively extracted with petroleum ether, chloroform and *n*-butanol. The petroleum ether extract was repeatedly chromatographed on silica gel column to give compounds **4**, **8** and **9**. From the *n*-butanol extract, compounds **1**, **2**, **3**, **5**, **6** and **7** were isolated.

Compound 1 was obtained as a yellow powder, showed UV absorption at 213, 257.5, 270 and 350 nm; Its IR spectrum show that there are phenol groups (3300 cm^{-1}) and α,β -unsaturated carbonyl group (1650 , 1615 , 1575 and 1490 cm^{-1}) in its structure. Form the chemical shifts of ^1H and ^{13}C NMR spectra, it could be considered as a C-glucosyl flavonoid. Comparing its ^{13}C NMR spectrum with those of soaflinetin (**5**, **7**, $3'$, $4'$, $5'$ -pentahydroxyl-6-C- β -D-glucopyranosyl flavone)^[2] and luteolin^[3] revealed that **1** has the same A-ring as isoafletin and the B-ring as luteolin. Therefore, the structure of **1** should be as 5,7,3',4',-tetrahydroxyl-6-C-glucopyranosyl flavone (isoorientin), a known C-glycosyl flavone. This compound is mainly distributed in the genus *Swertia* and *Gentiana* of the same family^[4-6]. And this is the first time to have isolated it from the genus of *Gentianella*.



Compound 2 was obtained as a yellow powder, UV, IR, ^1H and ^{13}C NMR spectra show that there are

a xanthone derivative with phenol groups, carbonyl group, a methoxyl and a glucosyl groups in its structure. Colour reaction with $\text{Pb}(\text{OAc})_2$ has not shown yellow revealed that it is not an ortho- and para-dihydroxyl xanthone. ^{13}C NMR spectrum shown that all of chemical shifts of **2** are fully overlapped with those of swertianolin^[7]. And acidic hydrolysis of **2** afforded bellidifolin(**10**)^[7] and glucose also revealed that **2** was swertianolin (8-O- β -glucopyranosyl bellidifolin). It has ever been isolated from *Gentiana campestris* L.^[8] and *Swertia japonica* Makino^[7] of the same family. And this the first time to have obtained it in the genus *Gentianella*.

Compound 3 was obtained as a white powder, mp. 101—104°C (dec.); $[\alpha]_{\text{D}}^{25}$ -62.10(c 0.248, MeOH), which UV absorption at 229 and 279 nm, and IR spectrum showed the presence of hydroxyl groups (3380 cm^{-1}) and aromatic rings (1600, 1505 cm^{-1}). According to ^1H and ^{13}C NMR spectra it were considered to be a mono-epoxylignan with 7-O-9' form. And all of the chemical shifts of **3** was fully overlapped with those of olivil 4'-O- β -D-glucoside^[9]. Thus, the structure of **3** was identified. As our knowledge, no lignans from gentianaceous plants have been investigated. This is the first report of isolation of **3** from the family of Gentianaceae.

In the ^1H and ^{13}C NMR spectra of compound **4**, common signals of a normal straight chain fatty alcohol were observed. Based on the biggest fragment ion peak of EI-MS spectrum were seen at m/z 421 $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$, suggesting the molecular formula to be $\text{C}_{30}\text{H}_{62}\text{O}$. So, compound **4** were determined to be n-1-triacontanol. This compound is widely existed in plant kingdom, and it is reported that is as a growth hormone could stimulate the plant growth and photosynthetic carbon metabolism in plant^[10-12], and it has the activity of preventing galactosamine-induced toxicity^[13].

To compare with authentic samples, compounds **5**—**7** were determined as iridoidal glycosides and to be assigned as known gentiopicroside (**5**)^[14], swertiamarin (**6**)^[15] and loganic acid (**7**)^[16] respectively. On the other two compounds, **8** as oleanolic acid and **9** is daucosterol. All of ^1H and ^{13}C NMR spectral data confirmed the identify.

It is noticed that the chemical constituents especially secoiridoidal glycosides, xanthone and flavonoid of *Gentianella azurea* are very similarity with those of *Gentiana* and *Swertia* which will be useful to expound the phylogenetic relationship between *Gentianella* and these two genera.

EXPERIMENT

MPS are uncorrected. Optical rotation were on a J-20C polarimeter. UV and IR spectra were measured on shimadzu UV-210A and IR-450 spectrophotometer, in MeOH and KBr pellets respectively. ^1H and ^{13}C NMR spectra were measured on a Bruker spectrosin A-400 spectrometer using TMS as internal standards. FAB-MS spectra were measured with JAB-HS mass spectrometer.

Plant material The whole herbs of *Gentianella azurea* (Bunge) Holub were collected at Ri-Ka-Ze of Xizang, China, by Prof. Jin-Shen Yang. The voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany.

Extraction and isolation Dried whole herbs of *Gentianella azurea* (575 g) were refluxed with MeOH. After removal of the solvent in vacuo, the residue (258 g) was suspended in H_2O and then successively extracted with petroleum ether, chloroform and n-Butanol. The petroleum ether was concentrated in vacuo to give a viscous residue (91 g), which was repeatedly chromatographed on silica gel column, eluting with

CHCl_3 -MeOH (60 : 1-10 : 1) to give **4** (35 mg) and fraction 1-9. Fr.1(6 g) was chromatographed on silica gel column, eluting with petroleum-acetone (10 : 1) to give **8** (155 mg); Fr.6(300 mg) was chromatographed on silica gel column, eluting with petroleum-acetone (10 : 1-3 : 1) to give **9** (22 mg). The *n*-Butanol layer was concentrated in vacuo to give a viscous residue (61 g), which was chromatographed on a silica gel column, eluting with CHCl_3 -MeOH- H_2O (40 : 10 : 1-10 : 10 : 1) to give fraction 1-5. Fr.2(26 g) which 2.1 g was repeatedly chromatographed Sephadex (eluting with 52% aq. MeOH) and silica gel column (eluting with CHCl_3 -MeOH, 15 : 1) to give **2** (370 mg), **5** (283 mg) and **6** (332 mg). Fr.3(6.89 g) was chromatographed on Sephadex (eluting with 52% aq. MeOH) and silica gel column (eluting with CHCl_3 -MeOH, 12 : 1) to afford **3** (75 mg). Fr. 4(7.2 g) was subjected to Sephadex (eluting with 55% aq. MeOH) and silica gel column (eluting with CHCl_3 -MeOH 7 : 1) to give **1** (700 mg) and **7**(246 mg).

Isorientin (1). yielding: 0.12%, a yellow powder, mp. 218—219°C (dec.). $\text{UV}\lambda_{\text{max}}\text{nm}(\log\epsilon)$: 213(4.54), 257.5(4.25), 270(4.26), 350(4.34); $\text{IR}\nu_{\text{max}}\text{cm}^{-1}$: 3300, 1650, 1615, 1575, 1490, 1450; EI-MS *m/z*: 430 $[\text{M}(\text{C}_{21}\text{H}_{20}\text{O}_{12})-\text{H}_2\text{O}]^+$, 412 $[\text{M}-2\text{H}_2\text{O}]^+$, 394 $[\text{M}-3\text{H}_2\text{O}]^+$, 299 $[\text{M}-\text{C}-\text{glu}+\text{CH}_2]^+$, 286 $[\text{M}-\text{C}-\text{glu}]^+$; ^1H NMR(DMSO): δ 7.32(1H, s, H-3), 7.33(1H, d, *J* = 7.0Hz, H-5), 6.87(1H, d, *J* = 7.0Hz, H-6), 6.49(1H, s, H-8), 6.44(1H, s, H-2'), 4.89(1H, d, *J* = 10Hz, H-1'); ^{13}C NMR(DMSO): 164.8(C-2), 103.9(C-3), 183.9(C-4), 161.9(C-5), 109.1(C-6), 166.2(C-7), 95.4(C-8), 158.7(C-9), 105.3(C-10), 123.6(C-1'), 114.2(C-2'), 147.0(C-3'), 151.0(C-4'), 116.6(C-6'), 75.4(C-1''), 71.8(C-2''), 80.2(C-3''), 72.8(C-4''), 82.6(C-5''), 62.9(C-6'').

Swertianolin (2). yielding: 0.77%, a yellow powder, mp. 251—254°C (dec.). $\text{UV}\lambda_{\text{max}}\text{nm}(\log\epsilon)$: 203(4.33), 217(4.21), 252(4.42), 275(4.13), 323.5(4.10), 362(3.56); $\text{IR}\nu_{\text{max}}\text{cm}^{-1}$: 3350, 1650, 1655, 1615, 1575, 1495, 1085, FAB-MS *m/z*: 459 $[\text{M}(\text{C}_{20}\text{H}_{22}\text{O}_{11})+\text{Na}]^+$, 436 $[\text{M}]^+$; ^1H NMR (DMSO): δ 6.31, 6.53(each s, H-2,4), 7.24, 7.11(each d, *J* = 9.0Hz, H-6,7), 3.88(3H, s, OCH_3), 4.79(1H, d, *J* = 7.6Hz, glu H-1'); ^{13}C NMR(DMSO): 162.7(C-1), 97.2(C-2), 166.3(C-3), 92.2(C-4), 156.4(C-4a), 145.1(C-10a), 141.1(C-5), 121.2(C-6), 112.5(C-7), 149.4(C-8), 112.0(C-8a), 181.1(C-9), 103.6(C-9a), 56.1(OCH_3), 103.3(C-1'), 73.6(C-2'), 76.1(C-3'), 69.9(C-4'), 77.4(C-5'), 60.9(C-6').

Olivil 4'-O- β -D-glucoside (3). yielding: 0.013%, a white powder, mp. 101—104°C (dec.). $[\alpha]_{\text{D}}^{25}$ -62.10 (c 0.248, MeOH); $\text{UV}\lambda_{\text{max}}\text{nm}(\log\epsilon)$: 204(4.74), 229(4.09), 279(3.61), $\text{IR}\nu_{\text{max}}\text{cm}^{-1}$: 3380, 1690, 1600, 1505, 1455, 1265, 1070, 1030; ^1H NMR (DMSO): δ 4.72(1H, d, *J* = 7.0Hz, $\text{H}_{\text{glu}}-1$), 7.14(1H, d, *J* = 2.0Hz, H-2), 7.09(1H, d, *J* = 8.0Hz, H-5), 6.87(1H, dd, *J* = 2.0, 8.0Hz, H-6'), 6.99(1H, d, *J* = 2.0Hz, H-2'), 6.75(1H, d, *J* = 8.0Hz, H-5'), 6.84(1H, dd, *J* = 2.0, 8.0Hz, H-6'), 3.41(3H, s, OCH_3), 3.31(3H, s, OCH_3); ^{13}C NMR (CD_3OD): 135.2(C-1), 111.6(C-2), 148.9(C-3), 147.1(C-4), 115.8(C-5), 120.7(C-6), 85.6(C-7), 61.8(C-8), 60.7(C-9), 134.9(C-1'), 116.2(C-2'), 150.3(C-3'), 146.5(C-4'), 116.2(C-5'), 124.0(C-6'), 40.6(C-7'), 82.4(C-8'), 77.8(C-9'), 56.8(OCH_3), 56.5(OCH_3), 102.9($\text{C}_{\text{glu}}-1$), 74.8($\text{C}_{\text{glu}}-2$), 77.8($\text{C}_{\text{glu}}-3$), 71.3($\text{C}_{\text{glu}}-4$), 78.0($\text{C}_{\text{glu}}-5$), 62.5($\text{C}_{\text{glu}}-6$).

***n*-1-Triacontanol (4).** Yielding: 0.0061%, a white solid, mp 77—78°C; EI-MS *m/z*: 421 $[\text{M}(\text{C}_{30}\text{H}_{62}\text{O})-\text{OH}]^+$, 393 $[\text{M}-\text{OH}-2\text{xCH}_2]^+$, 365 $[\text{M}-\text{OH}-4\text{xCH}_2]^+$, 337 $[\text{M}-\text{OH}-6\text{xCH}_2]^+$, ect. ^1H NMR (CD_3OD): δ 3.62(2H, q, *J* = 6.0 Hz, H-1), 0.85(3H, q, *J* = 6.0Hz, CH_3); ^{13}C NMR (CD_3OD): 63.1(C-1), 14.1(C-30), 32.9, 31.9, 29.7, 29.4, 29.4, 25.8, 22.7.

Gentiopicroside (5). yielding: 0.59%, a pale yellow powder. $\text{IR}\nu_{\text{max}}\text{cm}^{-1}$: 3400, 1700, 1610, 1435, 1275, 1075; ^1H NMR (CD_3OD): δ 7.45(1H, s, H-3), 4.65(1H, d, *J* = 8.0Hz, H-1'); ^{13}C NMR (CD_3OD):

98.6(C-1), 150.6(C-3), 105.0(C-4), 127.1(C-5), 117.1(C-6), 70.8(C-7), 135.0(C-8), 46.6(C-9), 118.5(C-10), 166.3(C-11), 100.2(C-1'), 74.6(C-2'), 78.0(C-3'), 71.6(C-4'), 78.4(C-5'), 62.8(C-6').

Swertiamarin (6). yielding 0.69%, a white powder, mp. 118—120°C (dec.). IR $_{\text{max}}$ cm $^{-1}$: 3400, 1695, 1615, 1410, 1275, 1075; ^1H NMR (CD_3OD): δ 5.73(1H, d, $J=1.3\text{Hz}$, H-1), 7.63(1H, s, H-3), 4.64(1H, d, $J=8.0\text{Hz}$, H-1'); ^{13}C NMR (CD_3OD): 99.2(C-1), 154.7(C-3), 109.1(C-4), 64.4(C-5), 33.8(C-6), 66.0(C-7), 133.8(C-8), 52.8(C-9), 121.2(C-10), 168.0(C-11), 100.2(C-1'), 74.5(C-2'), 77.9(C-3'), 71.5(C-4'), 78.6(C-5'), 62.7(C-6').

Loganic acid (7). yielding: 0.043%, a white powder. ^1H NMR (CD_3OD): δ 5.27(1H, d, $J=4.4\text{Hz}$, H-1), 7.39(1H, s, H-3), 3.09(1H, q, $J=8.1\text{Hz}$, H-5), 4.05(1H, m, H-7), 1.87(1H, m, H-8), 2.03(1H, m, H-9), 1.09(1H, d, $J=6.9\text{Hz}$, H-10), 4.66(1H, d, $J=7.8\text{Hz}$, H-1'); ^{13}C NMR (CD_3OD): 97.7(C-1), 152.0(C-3), 114.2(C-4), 32.1(C-5), 42.7(C-6), 75.0(C-7), 42.1(C-8), 46.6(C-9), 13.4(C-10), 170.9(C-11), 100.1(C-1'), 74.7(C-2'), 78.0(C-3'), 71.6(C-4'), 78.2(C-5'), 62.8(C-6').

Oleanolic acid (8). yielding: 0.027%, a white powder, mp. 312—314°C (dec.). ^{13}C NMR (CD_3OD): δ 39.4(C-1), 28.1(C-2), 78.2(C-3), 39.1(C-4), 55.9(C-5), 18.9(C-6), 33.3(C-7), 39.9(C-8), 48.2(C-9), 37.5(C-10), 23.9(C-11), 122.6(C-12), 144.9(C-13), 42.3(C-14), 28.4(C-15), 23.9(C-16), 46.6(C-17), 42.1(C-18), 46.8(C-19), 31.0(C-20), 34.3(C-21), 33.3(C-22), 23.8(C-23), 16.6(C-24), 15.5(C-25), 17.5(C-26), 26.2(C-27), 180.2(C-28), 33.3(C-29), 23.8(C-30).

Daucosterol (9). yielding. 0.0038%, a white amorphous powder, mp. 294—296°C (dec.). ^{13}C NMR (CD_3OD): δ 37.6(C-1), 28.6(C-2), 78.3(C-3), 39.4(C-4), 141.1(C-5), 122.0(C-6), 32.2(C-7), 32.2(C-8), 50.5(C-9), 37.0(C-10), 21.4(C-11), 40.1(C-12), 42.6(C-13), 56.4(C-14), 26.6(C-15), 28.6(C-16), 57.0(C-17), 12.1(C-18), 12.2(C-19), 36.5(C-20), 19.1(C-21), 34.4(C-22), 24.6(C-23), 46.2(C-24), 29.6(C-25), 19.5(C-26), 19.3(C-27), 23.5(C-28), 20.0(C-29), 102.7(C-1'), 75.4(C-2'), 78.7(C-3'), 71.9(C-4'), 78.4(C-5'), 63.0(C-6').

Acidic hydrolysis of 2. A solution of **2** (20 mg) in 10% $\text{H}_2\text{SO}_4/\text{H}_2\text{O}-\text{MeOH}$ (3 : 1)(10 ml) was refluxed for 3 h and the reaction mixture was extracted with Et_2O . The EtOH layer was washed with H_2O and then concentrated to dryness and the residue was recrystallized from MeOH to give **10** (5mg), mp. 276—279°C, which was identified by comparison of the ^1H and ^{13}C NMR spectra with those of authentic sample^[4]. The aqueous layer was neutralized with Amberlite MB-3 and concentrated to dryness. In the residue, glucose was identified by TLC (silica gel G; solvent system: iso- $\text{PrOH}-1/30\text{M H}_3\text{BO}_3$ (85 : 15); detected by spraying it with aniline-phthalate followed by heating. Rf: 0.62).

Bellidifolin (10). a yellowish needle crystal. mp. 276—279°C; EI-MS: 275[M($\text{C}_{14}\text{H}_{12}\text{O}_5+\text{H}$) $^+$]; ^1H NMR (DMSO): δ 6.59, 6.38(each s, H-2,4), 7.24, 6.63(each d, $J=9.0\text{Hz}$, H-6,7), 3.88(3H, s, OCH_3); ^{13}C NMR (DMSO): 161.9(C-1), 97.4(C-2), 167.0(C-3), 92.9(C-4), 157.3(C-4a), 143.3(C-10a), 137.3(C-5), 123.8(C-6), 109.5(C-7), 151.8(C-8), 107.5(C-8a), 184.0(C-9), 102.1(C-9a), 56.2(OCH_3).

Acknowledgement We are grateful to Prof Jin-Shen Yang for collecting plant materials, Prof. Qing-Chun Li (Yunnan University, China) for performing EI-MS analysis, Dr Bing-Jin-Li for the measurement of $[\alpha]_D$, and instrument group of our Laboratory for measuring NMR, IR and EI-MS spectra.

Reference

- [1] Ho T N. *Florae Reipublicae Popularis Sinicae. Flora* (62). Beijing: Sciences Press, 1988. 317.
- [2] Markham K R. *Techniques of Flavonoid Identification*. Academic Press, 1982. 85.
- [3] Chari V M, Wagner H. Recent adv. *Phytochemistry*, 1977, 12: 29.
- [4] Komatsu M. Studies on the constituents of *Swertia japonica* III. On the flavonoid constituents of the plants of *Swertia* spp. *Yakugaku Zasshi*, 1968, 88: 832.
- [5] Oyuungerel Z, Komissarenko N F, Batyuk V S *et al.* C-flavonoid glycosides of *Gentiana algida* Pall. *Khim.—Farm Zh*, 1984, 18(8): 967.
- [6] Chulia A J. Nouvelles C-glucosylflavones chez *Gentiana pedicellata*. *J Nat Prod*, 1985, 48(3): 480.
- [7] Sakamoto I, Tanaka T, Tanaka O *et al.* Xanthone glucosides of *Swertia japonica* Makino and related plant: Structure of a new glucoside. isoswertianolin and structure revision of swertianolin and norswertianolin. *Chem Pharm Bull*, 1982, 30(11): 4088.
- [8] Kaldas M, Hostettmann K, J-guillarmod A. Contribution a la phytochimie du genre *Gentiana* IX. Etude de composés flavoniques ex xanthoniques dans les feuilles de *Gentiana campestris* L. Iere communication. *Helv. Chim Acta*, 1974, 57: 2557.
- [9] Abe F, Yamauchi T, Wan A S C. Lignans related to Olivil from genus *Cerbera* (*Cerbera*. VI). *Chem Pharm Bull*, 1988, 36(2): 795.
- [10] Houtz R L. Stimulation of growth and photosynthetic carbon metabolism in *Chlamydomonas reinhardtii* with triacontanol. *Diss Abstr Int B*, 1986, 46(5): 1411.
- [11] Ries S K. Regulation of plant growth with triacontanol. *CRC crit Rev plant Sci*, 1985, 2(3): 239.
- [12] Vasudeva N. Effect of hormonol, miraculan and agronaa on yield of Arabica coffee. *J Coffee Res*, 1984, 14(4): 171.
- [13] Syamasundar K V, Singh B, Thakur R S *et al.* Antihepatotoxic principles of *Phyllanthus niruri* herbs. *J Ethnopharmacol*, 1985, 14(1): 41.
- [14] Inouye H, Yoshida T, Nakamura Y *et al.* Die stereochemie einiger secoiridoidglucoside und die revision der struktur des gentiopicrosides. *Tetrahedron Lett*, 1968, 42: 4429.
- [15] Inouye H, Wagner H, Horhammer L. *Pharmacognosy and Phytochemistry*. Springer-Verlag, New York: N. T., 1971, 290.
- [16] Ma W G, Wang D Z, Zeng Y L *et al.* Iridoidal glycosides from *Triplostegia grandiflora*. *Acta Botanica Yunnanica*, 1992, 14(1): 92.